Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 337 (2007) 210–218
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Stabilization of the maleate salt of a basic drug by adjustment of microenvironmental pH in solid dosage form

Erika A. Zannou, Qin Ji, Yatindra M. Joshi, Abu T.M. Serajuddin *

Pharmaceutical and Analytical Development Department, Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ 07936-7329, United States

Received 2 October 2006; received in revised form 22 December 2006; accepted 3 January 2007
Available online 9 January 2007

Abstract

Tablet formulations of the maleate salt of a basic drug (**I**) showed a major loss in potency and a lack of mass balance upon storage under accelerated stability testing conditions. No such stability issues were observed in capsules that were compositionally similar, and even the tablet was stable when it was encapsulated in capsule shell. It was identified that the salt converts to its free base form in the microenvironment of the tablet formulation. Studies using radiolabeled drug substance showed that the free base formed in the tablet volatilized under test conditions used and was absorbed in the wall of plastic container. No mass loss was observed with encapsulated tablets since the capsule shell either protected the drug substance from volatilization or trapped any drug substance that volatilized. The conversion of the salt to free base could be related to the pH-solubility profile of the compound where the pH_{max} (pH of maximum solubility) was 3.3–3.6, above which the salt would convert to base while no such conversion would occur below this pH. The microenvironmental pH of the tablet was found to be 4.3, favoring the salt-to-base conversion. A stable tablet formulation with shelf-life >3 years was successfully developed by lowering the microenvironemental pH of tablet from 4.3 to <3.0 by adding citric acid to the formulation.

Keywords: Salt; Base; Microenvironmental pH; Volatilization; Mass balance; Stability

1. Introduction

The maleate salt of a basic drug (dibenzo [b,f)oxepen-10-ylmethyl-methyl-prop-2-ynyl-amine hydrogen maleate (**I**) was developed for oral administration in the treatment of neurodegenerative disorders, such as Parkinson's disease and amylotrophic lateral sclerosis (Sagot et al., 2000; Waldmeier et al., 2000; Perentes et al., 2002). Hard gelatin capsules of 0.25-, 2.5- and 10-mg potencies were developed for phase I clinical studies, where the 2.5- and 10-mg capsules had acceptable shelf-life (<5% potency loss) at controlled room temperature for at least 2 years, and the 0.25-mg capsule required refrigeration (2–8 °C) to achieve a 2-year shelf-life. A complete mass balance of drug content and degradation products was obtained during the accelerated stability testing of capsules under various conditions, and degradation pathways were reported (Pan et al., 2006). Following initial clinical testing (Phases I and IIA) using capsules, it was decided to switch dosage form from capsule to tablet with potencies of 0.5, 2.5 and 10 mg for phases IIB and III clinical studies and ultimately for commercialization. The tablets were developed with a minimal change in composition from the capsule formulation to maintain continuity between developmental activities of two dosage forms and to ensure that their stability would be similar. Also, since a dose-dependent stability of capsule was observed, the lowest strength was increased from 0.25 mg in capsule to 0.5 mg in tablet with the expectation that this would increase the stability of formulation. However, surprisingly, it was observed that drug in tablets degraded much more rapidly than that in capsules, especially at high temperature and humidity. For example, there was >10% loss in potency when the 0.5-mg tablet was stored in induction-sealed and tightly capped high density polyethylene (HDPE) bottles at 40 °C/75% RH for 6 weeks. Additionally, there was no mass balance of drug content in the degraded tablets. On the other hand, no significant loss in potency and no mass balance issue were observed when a 0.25-mg capsule with similar composition to the tablet was prepared and subjected to the similar storage condition. The present study was undertaken

* Corresponding author. Tel.: +1 862 778 3995; fax: +1 973 781 8487.
E-mail address: abu.serajuddin@novartis.com (A.T.M. Serajuddin).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.01.005

to identify the cause of unexpected degradation of 0.5-mg tablets and to develop a stable tablet dosage form.

2. Materials and methods

2.1. Materials

Drug substance **I** (maleate salt) and its ^{14}C-radiolabeled and the free base forms were synthesized by Novartis Pharmaceuticals Corp. Analytical reagents purchased from different suppliers were used as received: acetonitrile (HPLC grade), Fisher Scientific (Fair Lawn, NJ); trifluoroacetic acid (TFA), sodium chloride (NaCl) and sodium hydroxide (NaOH), Aldrich Chemical Co. (Milwaukee, WI). The following excipients that had been approved for use in pharmaceutical dosage forms were received from the inventory of Novartis' commercial manufacturing facility: maize starch, lactose, mannitol, microcrystalline cellulose (diluents), polyvinylpyrrolidone (binder), crospovidone (disintegrant), colloidal silicon dioxide (glidant), magnesium stearate and hydrogenated castor oil (lubricants). Maleic acid and citric acid, which were used as pH-modifiers in formulations, were purchased from Sigma (St. Louis, MO).

2.2. Methods

2.2.1. pH-solubility profile

The pH-solubility profile over the range of pH 1–9 was generated at 25 °C by using both maleate salt and free base form of **I**. Excess amounts of solid drug substance were added to purified water. The suspensions were placed at 25 ± 1 °C in a water bath and shaken using a Wrist Action® shaker (Burrel Corp., Pittsburgh, PA) for 3 h. The suspensions were sampled and the pH was measured by potentiometry using Accumet® AR15 pH meter (Fischer Scientific, Fair Lawn, NJ). The pH of suspensions was readjusted with maleic acid or NaOH as needed and equilibrated for three more hours. The suspensions were then centrifuged and the supernatant liquids were filtered through Acrodisc® Gelman® PTFE filter of 0.20-μm pore size (Fischer Scientific, Fair Lawn, NJ). It was previously validated that the filter did not adsorb drug. Filtrates were diluted and analyzed by HPLC using the method described later. The undissolved material from the centrifuge tube was filtered on a No. 1 Whatman paper filter (Whatman International, England), rinsed with purified water and allowed to dry overnight at room temperature. The dried solids were analyzed by differential scanning calorimetry (DSC 2920, TA Instruments, New Castle, DE) in sealed aluminum pans at 10 °C/min from 20 to 180 °C. The melting point was determined as the extrapolated onset temperature of the melting endotherm and was compared to the melting point obtained for the pure drug substances (maleate salt and free base) under the same conditions.

2.2.2. pH-degradation rate profile

The solution stability of **I** at a concentration of 0.06 mg/mL was evaluated at 70 °C as a function of pH using maleate (pH 2.0) and citrate (pH 3.5, 4.5 and 6.0) buffer systems; 20% acetonitrile was added to the pH 6 buffer to ensure that the drug remained in solution. Ionic strength was maintained at 0.1 M using NaCl. Solutions were placed in crimped glass vials and sampled at predefined intervals. No precipitate was observed throughout the study. Samples were analyzed by HPLC for drug and degradation products. Degradation rate constants were calculated at various pHs assuming pseudo-first-order degradation kinetics.

2.2.3. Preparation of dosage forms

Capsules with dosage strengths from 0.25 to 10 mg (free base equivalent) and tablets with dosage strengths from 0.5 to 10 mg (free base equivalent) were prepared. Compositions of selected formulations with concentration of each component present (wt%) are shown in Table 1. A wet granulation process was applied to the manufacture of capsule, where, for example, a 0.25-mg capsule (Formulation 1) had a fill weight of 160 mg and contained 0.35 mg of **I** (maleate salt), lactose and maize starch as diluents, PVP as the binder, crospovidone as the disintegrant, colloidal SiO_2 as the glidant and magnesium stearate as the lubricant. Both wet granulation and direct compression processes were used for tablets, with formulations compositionally

Table 1
Compositions of selected formulations shown with concentration (w/w%) of each component

Ingredients	1 (capsule, 0.25 mg, WG)	2 (tablet, 0.5 mg, WG)	3 (tablet, 0.5 mg, DC)	4 (tablet, 0.5 mg, DC)	5 (tablet, 0.5 mg, WG)
Compound **I** maleate salt	0.22	0.71	0.71	0.71	0.55
Maize starch	15.62			18.60	
Lactose	69.29	72.00	74.39	74.39	
Mannitol					71.95
Microcrystalline cellulose		18.00	18.60		15.00
Polyvinyl pyrrolidone	3.75	5.00			2.00
Citric acid					2.00
Crospovidone	10.0	3.29	5.00	5.00	4.00
colloidal SiO_2	0.62		0.30	0.30	
Magnesium stearate	0.50	1.00	1.00	1.00	
Hydrogenated castor oil					4.50
Total weight/unit (mg)	160	100	100	100	130

WG, wet granulation; DC, direct compression.

similar to capsules except that the total weight ranged between 100 and 130 mg depending on dose; the tablet weight for the 0.5-mg potency was 100 or 130 mg. Additionally, maize starch was replaced by microcrystalline cellulose in most tablet formulations (Formulations 2–5). In certain tablet formulations, lactose was replaced by mannitol as diluent and magnesium stearate (∼1%) was replaced by hydrogenated castor oil as the lubricant to further optimize formulations (for example, Formulation 5). Powder mixtures for direct compression tablets were prepared through successive screening and blending of the drug substance and excipients. Wet granulations for both capsules and tablets were prepared using a high shear granulator with top blade (Collette Gral, Wommelgem, Belgium). For this purpose, screened drug substance, diluent, binder and half of the needed disintegrant were blended dry and then purified water (22.5%) was added as the granulation fluid. The wet granules were dried with a 50 °C inlet temperature either on trays or in a fluid bed drier depending on the manufacturing scale. The drying process did not have any significant impact on physical properties of granules (loss on drying, particle size distribution, flow) and tablets (hardness, friability, disintegration time). The granules were sized and blended with the screened external components (the other half of the disintegrant and the glidant, when applicable). Finally, the blends were lubricated with magnesium stearate or hydrogenated castor oil. When a pH-modifier (maleic acid, citric acid or NaOH) was added to the formulation, it was added in solution in the granulation fluid.

Capsules were filled using an H&K encapsulator (Bosch, Germany). Tablets were compressed using a multi-station rotary press (Manesty Compacting, Liverpool, UK).

Capsules and tablets were packaged and placed on stability as per conditions detailed in Section 3. Initial assay and accelerated stability data were obtained through HPLC analysis as detailed later.

2.2.4. Measurement of microenvironmental pH

Slurries of excipients, drug substance or formulations were prepared in polypropylene tubes (Corning Inc., Corning, NY). A known amount of solid, approximately 50–100% in excess of the amount needed to saturate the medium, was added to purified water and mixed for 15–30 min. Equilibrium pH was achieved in all cases within 15 min of vortexing indicating that aqueous phases of suspensions were nearly saturated, at least in relevance to their pH. The pH of suspensions was measured by potentiometry using an Accumet® Research AR15 pH meter equipped with an Accumet® glass electrode (Fisher Scientific, Pittsburgh, PA).

The same measurement was used to determine the target amount of pH-modifier needed in the formulation to ensure a specific microenvironmental pH. The suspensions of 0.5-mg formulations were titrated using maleic acid or citric acid solutions and pH of slurries were measured as a function of the amount of acid added.

2.2.5. HPLC method

A 2690 Alliance HPLC system (Waters, Milford, MA) was used in this study. The separation was achieved using a Waters YMC™ ODS-AQ, 4.6 mm × 50 mm, column with particle size of 3.0 μm and pore size of 120 Å (Waters, Milford, MA). Samples were sonicated and shaken in the presence of a 60:40 (v/v) water–acetonitrile extraction solvent and then centrifuged. The supernatant was analyzed for drug and degradation products using a gradient system with UV detection at 281 nm (996 PDA diode array detector, Waters, Milford, MA). The mobile phase A contained 0.1% TFA and the mobile phase B contained acetonitrile. Similar HPLC conditions were reported previously (Pan et al., 2006).

2.2.6. Radiolabel study

A blend of ^{14}C-radiolabeled **I** drug substance (maleate salt) and various excipients was prepared according to a 0.5-mg tablet formulation. The radiolabeled blend samples were packaged in two HDPE and amber glass bottles, and the bottles were then closed with induction seal using child resistant caps. The bottles were stored at 50 °C for 1 month. The 60:40 (v/v) water–acetonitrile solvent was used to extract samples and thoroughly wash the insides of bottles. The extraction and washing suspensions were combined, centrifuged and assayed by HPLC (Waters 600E Pump, 717 Autosampler, Waters, Milford, MA). Drug substance **I** was detected in test solution both using a 996 photo diode array UV-detector and a Ramona 90 radioactive Flo-thru monitor (Waters, Milford, MA).

A Ludlum Geiger counter model C-1 (Sweetwater, TX) with a 44-7 probe was used to measure any radioactivity adsorbed onto the inside and the outside surfaces of the bottles, and the measurements were performed in triplicate. Furthermore, two pieces of plastic were sliced from the outside surface of HDPE bottle, one from the top and the other from the bottom, and they were tested separately for radioactivity using the Geiger counter.

3. Results and discussion

3.1. Drug substance properties

The free base form of **I** has a pK_a of 6.3, a MW of 275 and a melting point of 74.7 °C, and it exhibits poor aqueous intrinsic solubility (about 2 μg/mL at 25 °C, pH 7.9). During form selection, the need for a salt was identified to improve the drug physical properties (e.g., increase mp) and dissolution rate (Morris et al., 1994). The maleate salt was identified as the salt of choice for development based on ease of synthesis, physicochemical properties and biopharmaceutical considerations. The structure of **I** (maleate salt) is shown in Fig. 1. It is a crystalline non-hygroscopic solid (162.9 °C) that is physically and chemically stable at various storage conditions, including 4 weeks at 80 °C in open glass bottle, and 6 weeks at 50 °C with 20% added water in closed glass bottle, and 3 years at 25 °C/60% RH in bulk.

The pH-solubility profile of the maleate salt is shown in Fig. 2. The aqueous solubility of salt at equilibrium with purified water is 1.5 mg/mL at 25 °C with a pH of 3.5. The measured solubility as a function of pH matches the theoretical solubility calculated based on the pK_a (6.3) and intrinsic solubility (2 μg/mL) in the

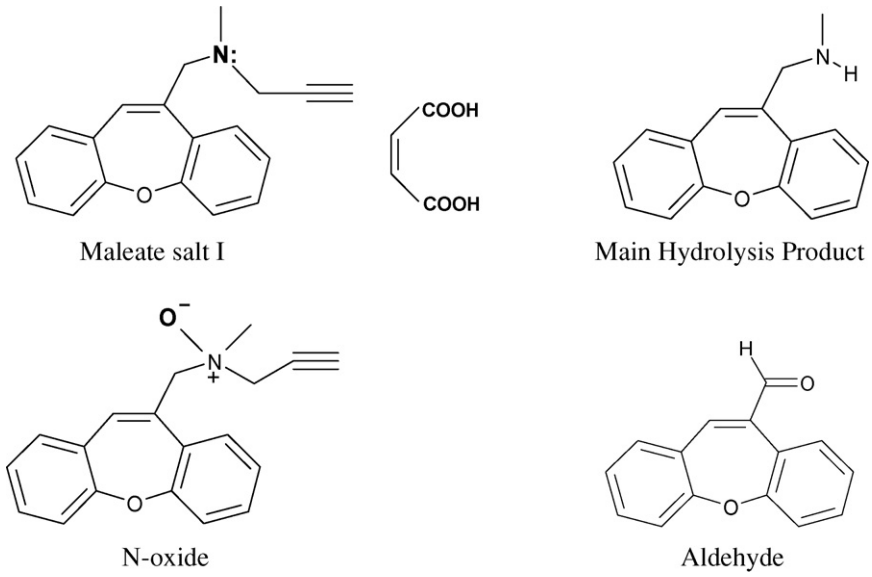

Fig. 1. Chemical structure of **I** and its degradation products.

pH range, where the solid phase is the free base (pH > 3.6). In the pH range, where the solid phase is the maleate salt (pH < 3.3), a decrease in solubility is observed due to common ion effect. The pH range between 3.3 and 3.6 has been identified as the pH_{max} region (Pudipeddi et al., 2001), above which the maleate salt converts to the free base with increasing pH; the solid phase was characterized by DSC to be a mixture of maleate salt and free base in the pH range of 3.3–3.6.

The pH-degradation rate profile of **I** is shown in Fig. 3, where drug substance exhibited pH-dependent stability with significant increase in degradation with increasing pH. The degradation rates at pH 3.5, 4.5 and 6.0 were 3, 12 and 15 times faster, respectively, than the degradation rate at pH 2. The main degradation product identified was a hydrolysis product shown in Fig. 1. As will be discussed later, the hydrolysis product was also the main degradation product observed in solid state. Two oxidative pathways were also identified in solid state for this compound (Pan et al., 2006); however, they were not detected in a significant extent in tablet or capsule formulations and were, therefore, outside of the scope of the present publication.

3.2. Dosage form stability

As mentioned earlier, dosage forms were developed in multiple strengths ranging from 0.25 to 10 mg for capsule and 0.5–10 mg for tablets, and their stability was evaluated by packaging 30 capsules or tablets per 30-cm^3 HDPE bottle with induction seal and subjecting them to accelerated test conditions. Since the maximum degradation was observed at the lowest strength of each dosage form, issues related to the stability of only 0.25-mg capsule and the 0.5-mg tablet are reported here. Direct comparison of the same low dose in capsule and tablet was not feasible since two specific doses were selected for development. However, as detailed later on, chemical stability for a compound that is prone to hydrolytic degradation is expected to be worse in the most diluted formulation when stored under similar conditions. Thus, the 0.25-mg capsule would be expected to exhibit a poorer stability profile than the 0.5-mg tablet. In addition, even for the same dose, a formulation would be expected to be less stable in a capsule than in a tablet due to the presence of a significant amount of moisture in the capsule shell.

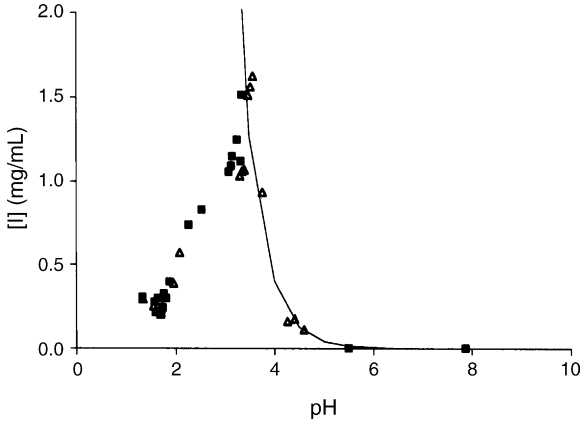

Fig. 2. pH solubility profile of drug substance **I**: theoretical (−), and with free base (■) and maleate salt (△) as starting materials.

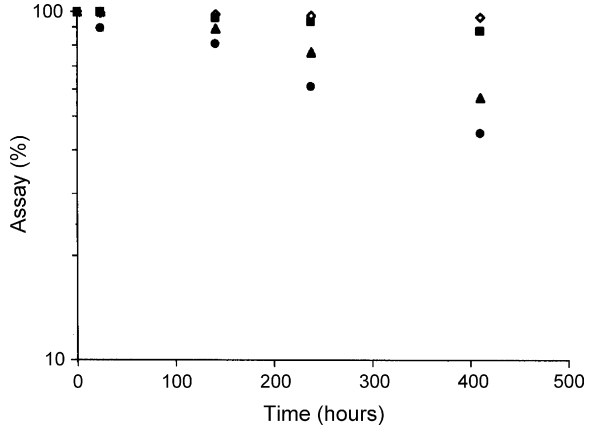

Fig. 3. pH stability profile of **I** at pH 2.0 (◊), 3.5 (■), 4.5 (▲) and 6.0 (●).

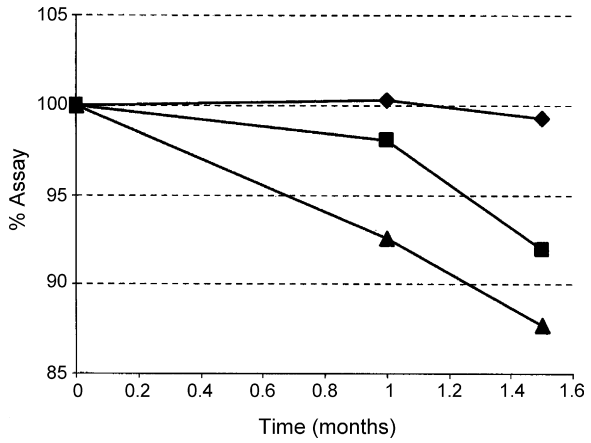

Fig. 4. Formulation stability of **I** in HDPE bottles with induction seal at 40 °C/75% RH: 0.25 mg capsule (♦), 0.5 mg wet granulated tablet (■) and 0.5 mg direct compression tablet DC (▲).

The wet-granulated 0.25-mg capsule formulation was used in phase I clinical studies, and two prototype 0.5-mg tablet formulations (Table 1, Formulations 2 and 3) were developed by wet granulation and direct compression, respectively. Any changes in composition from capsule to tablet was aimed at decreasing the potential for water adsorption by excipients to reduce drug hydrolysis in solid state (elimination of maize starch and decrease of crospovidone) and imparting better tabletting properties (addition of microcrystalline cellulose). Fig. 4 shows the stability profiles of the formulations at 40 °C/75% RH as a function of time for up to 1.5 months, where it is observed that tablets, whether manufactured by direct compression or wet granulation, lost their potency rapidly while the capsule formulation was stable. Moreover, the loss in potency in tablets was not accompanied by corresponding increase in degradation products in HPLC chromatograms; in other words, there was no mass balance. In contrast, there was no loss in potency of capsules and a full mass balance was observed after they were stored at 40 °C/75% RH for the same period of time. As shown in Table 1, the concentration of **I** (maleate salt) in 0.5-mg tablets (Formulations 2 and 3) was over three times higher than that in the 0.25-mg capsule (Formulation 1). This is because the capsule had a lower strength (0.25-mg versus 0.5-mg) and a higher fill weight (160-mg versus 100-mg) as compared to the tablet. Since the product stability for both capsules and tablets improved with higher ratios of drug to total weight in the higher-strength formulations, it was expected that the tablet would have better stability than the capsule. Although the appearance of any chemical degradation products in the two low-strength capsule and tablet was very low, the tablets showed high assay/mass loss under similar stability testing conditions.

Extensive studies were undertaken to explore and understand whether the mass balance issue of tablets could be related to analytical methods. Different extraction media and conditions to ensure complete extraction as well as different HPLC methods and detection systems to ensure full separation of active and potential degradation products were investigated. However, none of these could explain the decrease in drug potency in tablets.

3.3. Identification of the stability problem

3.3.1. Effect of encapsulation on tablet stability

In addition to the potency, the primary difference between the compositions of capsule and tablet was that in the tablet, maize starch was replaced by microcrystalline cellulose. Question was asked: Could the presence of microcrystalline cellulose be responsible for the loss in tablet potency? For this reason, another tablet formulation was developed by using maize starch in place of microcrystalline cellulose (Formulation 4). To mimic capsules, the tablets were also filled in hard gelatin capsules. Both encapsulated and unencapsulated tablets packaged in amber glass and HDPE bottles were evaluated for stability at high temperature and humidity conditions (40 °C/75% RH) for up to 6 weeks, and the results are shown in Fig. 5. It was very interesting to note that there was no loss in potency from encapsulated tablets, while over 15% loss in potency was observed from the unencapsulated tablets stored in HDPE bottles. These results then suggested that unencapsulated tablets were losing active drug substance without accounting for the entire mass. With these results it appeared more and more likely that the drug was being lost by volatilization from the unencapsulated tablets. It was hypothesized that an equilibrium between the solid and vapor phase in the small volume of the capsule may not be allowing further volatilization of drug from the tablet, thus accounting for minimal to no loss as compared to the tablet. It was also evident that there was a significant effect of packaging on the stability of tablets; the loss in potency of unencapsulated tablets was higher in the HDPE bottles as compared to the glass bottles, as shown in Fig. 5, which could be due to a higher permeability of HDPE material (compared to glass) and possible interaction between **I** (maleate salt) and the packaging material (HDPE). Solution stability of **I** was conducted at 40 °C in two glass vials: one vial with HDPE material (cut pieces of HDPE bottles) submerged in 20 mL of a 0.2 mg/mL solution of **I** in a 20 mM pH 4 citrate buffer at 60 °C for 4 weeks and the other one without HDPE material but otherwise under similar condition. The stability results in Fig. 6 showed that there was a significant loss in mass (about 60%) in presence of HDPE material, while there was practically no mass loss in the solution without the HDPE material. These results indicated that HDPE could be contributing to the loss of drug by sorbing the drug into its

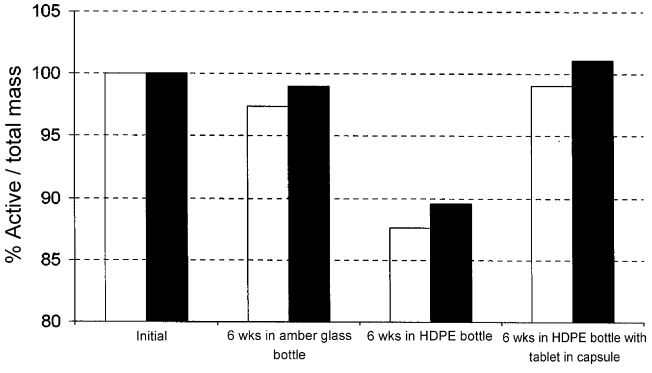

Fig. 5. Stability of 0.5-mg tablet (Formulation 4) at 40 °C/75% RH for 6 weeks in different packaging: % active (clear) and % total mass (black).

Table 2
Testing results for [^{14}C] radiolabeled **I** stability samples at 50 °C for 1 month

Packaging	Recovery by HPLC	Radioactivity[c] inside empty bottle	Radioactivity[c] outside empty bottle	Estimated total mass
Amber glass bottle	91%	3000 CPM (9%[a])	0 CPM	100%
HDPE bottle	56%	13,000 CPM (39%[b])	2000 CPM (6%[b])	101%

[a] Assuming 3000CPM is corresponding to 9% mass loss measured by HPLC.
[b] Estimated values based on the above assumption.
[c] Measured by Geiger counter.

permeable matrix, thus facilitating further volatilization of the drug from the tablet, resulting in significant mass loss in the unencapsulated tablet in the HDPE bottle.

3.3.2. Volatilization of drug

Further studies were conducted to confirm that the drug indeed volatilized from the tablets. A powder blend with composition similar to directly compressed 0.5-mg tablet prepared by using radiolabeled **I** was stored in an amber glass bottle and a HDPE bottle at 50 °C for 1 month. The blends in the bottles were assayed using a HPLC system, and a Geiger counter was used to detect the presence of radiolabeled material in the empty bottles. As shown in Table 2, a significantly higher mass loss (44%) was observed for the blend sample in the HDPE bottle as compared to 9% loss in the glass bottle. Substantial amounts of radioactivity were still detected on the inner surface of both empty glass (3000 CPM) and HPDE (13,000 CPM) bottles, the amount in the plastic bottle being over four times higher. It was surprising that radioactivity (2000 CPM) was observed even on the outer surface of the empty plastic bottle. From these results, it was apparent that there was a qualitative correlation between approximately five times difference in the loss in drug potency in glass versus plastic bottles and over four times difference in radioactivity on inner surfaces of glass versus plastic bottles. This also agrees with the hypothesis of the volatilization of **I** from tablets. It is possible that the vapor of **I** could migrate into the plastic and further facilitate volatilization and mass loss of **I** from the HDPE bottle. Analysis of the Geiger counter data indicated that the radioactivity on the outer surface of the HDPE bottle was only detected in the bottom part of the bottle, suggesting that the **I** vapor was heavier than air. The effect of volatilization had profound effect on the stability of the lower-potency tablets, because a loss of only 0.05 mg of material was needed to show a 10% loss in potency of the 0.5-mg tablet, whereas for higher doses much higher amounts had to be lost to show significant mass loss.

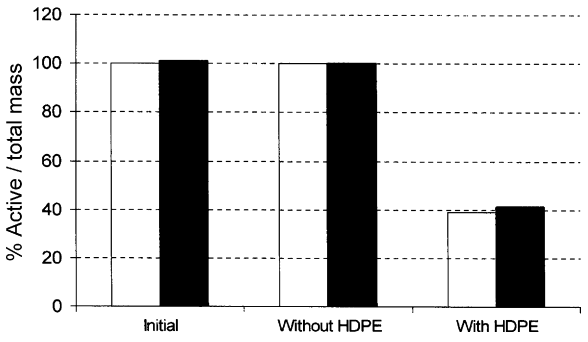

Fig. 6. Stability of 0.2 mg/mL solution **I** for 4 weeks in pH 4 citrate buffer (20 mL) at 40 °C/75% RH in glass vials with and without high density polyethylene (HDPE): % active (clear) and % total mass (black).

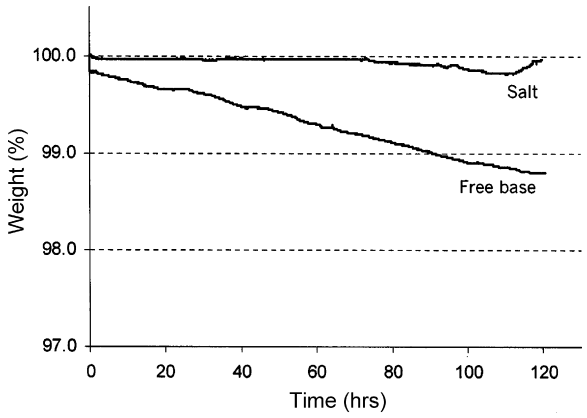

Fig. 7. TGA testing (holding at 60 °C for 5 days) for drug substance **I** maleate salt vs. **I** free base (6 mg each).

3.3.3. Effect of microenvironmental pH

Due to its high melting point, **I** (maleate salt) was not expected to be volatile. The free base, with a melting point of 74.7 °C, could be prone to volatilization as shown in Fig. 7. This could only be valid if the maleate salt would convert into the free base in the solid dosage form. Based on the pH-solubility profile and pH_{max} determination discussed earlier, a conversion of the maleate salt to the free base would occur above pH_{max} at pH > 3.6. The slurry pH of various formulations and individual excipients used was thus measured (Table 3) and was

Table 3
Slurry pH of drug substance **I** formulations and various excipients

Materials	Microenvironmental pH
Neat drug substance **I** maleate salt	3.5
Capsule formulation (0.25 mg)	5.0
Tablet formulation (0.5 mg)	4.2
Tablet formulation (10 mg)	4.1
Placebo tablet formulation (no drug substance)	5.9
Lactose	6.1
Mannitol	4.7
Microcrystalline cellulose	4.7
PVP	3.7
Crospovidone	4.9
Colloidal SiO_2	5.2
Magnesium stearate	7.1
Maize starch	6.3
Hydrogenated castor oil	7.5

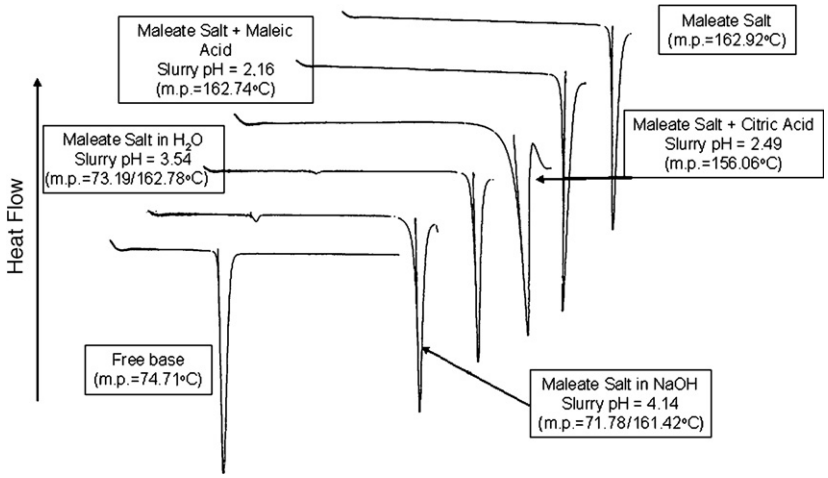

Fig. 8. DSC scans of drug substance **I** free base, maleate salt and the maleate salt mixtures with acid and base.

shown to be higher than the pH_{max}. For the higher strengths, the higher drug load does not have a significant effect on the formulation microenvironmental pH. Both 0.5- and 10-mg tablet formulations have a pH of about 4.1–4.2 (Table 3). The difference in stability between low and high strengths appears to be due to the difference in relative amounts of drug substance that would be converted from the maleate salt to the free base in the microenvironment of a tablet.

To confirm the working hypothesis, an experiment was conducted with pure drug substance of which microenvironmental pH was modified in slurry conditions. Three suspensions of maleate salt were titrated using pH-modifiers: with maleic or citric acid to lower the pH to between 2.2 and 2.5, and with NaOH to increase the slurry pH to 4.2 (equivalent to the 0.5-mg formulation slurry pH). After equilibration, the suspensions were filtered, rinsed and let dry overnight. The remaining solid was then analyzed by DSC and compared to the untreated free base and maleate salt as well as the solid from the aqueous maleate salt suspension (Fig. 8). As suggested by the working hypothesis and in accordance with the pH solubility profile, solid drug substance with a slurry pH at or higher than pH_{max} contained mainly maleate salt with a small amount of free base. The relative free base amount was higher at pH 4.2 compared to pH 3.5. At pH values lower than the pH_{max}, the solid drug substance consisted only of maleate salt.

The next step was to test if the conversion of drug substance to free base in the formulation would correlate with the observed stability, specifically the assay/mass loss. For this purpose, several drug-excipient blends at 100-g scale were prepared, where the composition of Blend A was similar to that of Formulation

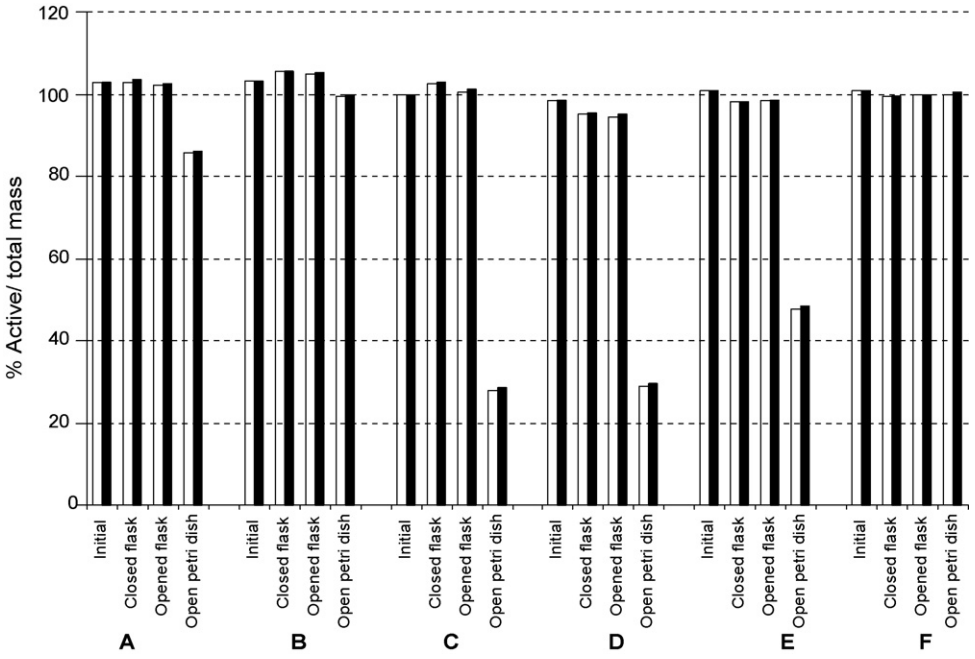

Fig. 9. Three-week stability result at 50 °C/75% RH in glassware for 0.5-mg wet granulation blends with different microenvironmental pH and drug form: % active (clear) and % total mass (black). (A) Tablet formulation 2, slurry pH of 4.2; (B) Formulation 2 granulated with citric acid, slurry pH of 2; (C) Formulation 2 granulated with NaOH, slurry of pH of ∼5; (D) Formulation 2 prepared with free base instead of maleate salt (pH 4.2); (E) drug substance **I** free base; (F) drug substance **I** maleate salt.

Table 4
One-year stability results for 0.5-mg compound **I** tablets (Formulation 5) in HDPE bottles with induction seals and silica bags (1-g bag per bottle)

Storage condition		% Assay	Total degradation product (%)	Total mass (%)
25 °C/60% RH	Initial	97.5	0.0	97.5
	3 months	96.5	0.06	96.6
	6 months	96.8	0.09	96.9
	9 months	98.3	0.11	98.4
	12 months	97.8	0.12	97.9
30 °C/70% RH	3 months	96.3	0.09	96.4
	6 months	96.8	0.14	96.9
	9 months	98.3	0.25	98.6
	12 months	96.9	0.40	97.3

Each 30-cm^3 bottle contains 90 tablets.

2 in Table 1 with a microenvironmental pH of ∼4.2, and Blends B and C were also similar, with the exception that citric acid and NaOH were used as pH-modifiers to adjust microenvironmental pH to 2 and pH ∼5, respectively. In addition, a similar blend with the adjusted microenvironmental pH of ∼4.2 was prepared with **I** free base instead of the maleate salt (Blend D). All blends were dried at ∼50 °C for 2–3 h to remove any water that was used during the adjustment of pH, and samples of uncompressed blends were stored in glassware of different shapes (open Petri dish versus closed 50 cm^3 volumetric flask versus open 50 cm^3 volumetric flask) and were placed at 50 °C/75% RH for 3 weeks. The different shapes for glassware were chosen to further emphasize the potential effect of volatilization in this degradation pathway. The 3-week stability results (Fig. 9) showed no assay/mass loss for Blends A (pH 4.2), B (pH 2), C (pH 5) and D (free base used, pH 4.2) in open and closed volumetric flasks. High assay/mass loss (∼70%) was observed for Blends C and D stored in open Petri dish, while the assay/mass loss for Blend A was lower (20%), and no assay/mass loss was observed for acidified Blend B. For comparison, similar stability studies were also conducted for **I** free base and maleate salt drug substances (Fig. 9). The stability behavior of the **I** maleate salt (F) was very similar to that of the acidified blend (B, pH 2), while the free base (E) stored on the Petri dish lost ∼45% of mass, confirming its volatilization.

Based on the understanding of the pH effect on **I** salt and free base (Figs. 2 and 8), one can adequately explain the stability behavior observed in Fig. 9. Since **I** free base vapor is heavier than air and it cannot migrate into the glass matrix (Fig. 5), the vapor concentrated at the bottom of glass volumetric flasks, and this prevented further volatilization when the equilibrium was reached between vapor and solid state. Therefore, there was no assay/mass loss for all the samples in open and closed volumetric flasks. On the other hand, in open Petri dish, **I** free base vapor could easily escape into air, thus driving further volatilization from **I** free base. The degree of volatilization was determined by the availability of **I** free base in the samples. Among all drug-excipient blends with different microenvironmental pH conditions used (Fig. 9), the amount of **I** free base was possibly the highest in C (pH 5) and D (the free base with pH 4.2), resulting in high assay/mass loss (∼70%) in open Petri dish, followed by about 20% assay/mass loss for Blend A (maleate salt with pH 4.2), and no assay/mass loss for acidified formulation (Blend B, pH 2).

3.4. Solution to the stability problem

It was clear from the above results that microenvironmental pH of formulations had to be adjusted below 3 to prevent drug loss due to volatilization. Based on a statistical design of experiments (DOE), several prototype tablet formulations were prepared by adjusting the microenvironmental pH to about pH 2 by adding different levels of maleic acid or citric acid. The levels of acid to be added to the formulation were determined by titrating a 30% slurry of the formulation with either maleic or citric acid until the slurry pH was lower than the pH_{max}. The pH-modifiers were added only using the wet granulation process, as it had been shown by Badawy et al. (1999) that it is more efficient than adding pH-modifiers in the dry state. In all cases, the tablets demonstrated satisfactory stability (<0.5% degradation) and complete mass balance when samples were stored in HDPE bottles at 40 °C/75% RH for a period of 6 weeks. A drug-excipient compatibility testing based on the model of Serajuddin et al. (1999) indicated that the adjustment of microenvironmental pH would also increase the chemical stability of the formulation, in general agreement with the pH-rate profile shown in Fig. 3. A prototype 0.5-mg tablet formulation optimized in this way (Table 1, Formulation 5) showed <0.5% degradation when in stored in HDPE bottles containing silica bags (30 tablets and one 1-g silica bag per 30-cm^3 bottle) at 30 °C/70% RH for 1 year (Table 4). The lubricant in Formulation 5 was changed from magnesium stearate to hydrogenated castor oil because the DOE indicated a slightly but significantly lower (∼0.1%, w/w) oxidative degradation (although no mass loss with both lubricants) when hydrogenated castor oil was used. Similarly, a slightly lower oxidative degradation was also the reason for using citric acid, instead of maleic acid, to adjust microenvironmental pH. Based on these results, the development of a 0.5-mg tablet with a 3-year shelf-life at controlled room temperature (extrapolated from data in Table 4) was feasible.

4. Conclusions

This study demonstrated the complex nature of the assay/mass loss problem involving different drug forms, dosage forms, packaging materials, microenvironmental pH, excipients, humidity and temperature. Systematic investigations were conducted to identify the cause of assay/mass loss for this

chemically stable basic drug in the tablet formulation: (1) the analytical methods were thoroughly evaluated to exclude the possibility of errors due to testing method; (2) then the stability study on different dosage forms (capsule versus tablet versus encapsulated tablet) showed stabilizing effect of the capsule shell which served as a barrier; (3) the radiolabel study indicated strong interaction between the drug and the HDPE packaging material; (4) the stability results of the blend formulations with different pH in different containers (open Petri dish, open and closed volumetric flasks) further pointed to the volatilization of **I** free base; (5) the study on salt to free base conversion as the function of microenvironmental pH confirmed that **I** maleate salt can be readily converted into its free base at pH higher than the pH_{max}. In summary, **I** maleate salt can be converted into its free base at pH 4.2 and the free base can sublime and easily migrate into the HDPE packaging material, thus further driving the volatilization process, resulting in additional assay/mass loss. In addition, pH stability profile and excipient compatibility study indicated that the chemical stability of the drug can be enhanced by controlling the formulation microenvironmental pH. Based on the effect of microenviromental pH on the physical and chemical stability of the drug, a stable 0.5-mg tablet with a shelf-life of 3 years as successfully developed by acidification of the formulation using 2% (w/w) citric acid.

Acknowledgements

The authors gratefully acknowledge Mr. Mark Mecadon for his contribution to the formulation and its stabilization, Dr. Amy Wu for her contribution to the radiolabel study, Dr. Frances Liu and Ms. Wei Wang for their contribution to the analytical method development and other assay support, and Mr. James Pazdan for his contribution to design of experiments and stability data analysis.

References

Badawy, S.I.F., Williams, R.C., Gilbert, D.L., 1999. Effect of different acids on solid-state stability of an ester prodrug of a IIb/IIIa glycoprotein receptor antagonist. Pharm. Dev. Technol. 4, 325–331.

Morris, K.R., Fakes, M.G., Newman, A.W., Thakur, A.B., Singh, A.K., Venit, J.J., Spagnuolo, C.J., Serajuddin, A.T.M., 1994. An integrated approach to the selection of optimal salt form for a new drug candidate. Int. J. Pharm. 105, 209–217.

Pan, C.K., Liu, F., Ji, Q., Wang, W., Drinkwater, D., Vivilecchia, R., 2006. The use of LC/MS, GC/MS, and LC/NMR hyphenated techniques to identify a drug degradation product in pharmaceutical development. J. Pharm. Biomed. Anal. 40, 581–590.

Perentes, E., Andringa, G., Waldmeier, P.C., Roth, D.R., Ibrahim, M., Cools, A.R., 2002. Neuroprotective effects of TCH346 in bilaterally MPTP-treated rhesus monkeys. J. Neuropathol. Exp. Neurol. 61, 461.

Pudipeddi, M., Serajuddin, A.T.M., Grant, D.J.W., Stahl, P.H., 2001. Solubility and dissolution of weak acids, bases, and salts. In: Stahl, P.H., Wermuth, C.G. (Eds.), Handbook of Pharmaceutical Salts—Properties, Selection, and Use. Verlag Helvetica Chimica Acta, Zurich, pp. 19–39.

Sagot, Y., Toni, N., Perrelet, D., Lurot, S., King, B., Rixner, H., Mattenberger, L., Waldmeier, P.C., Kato, A.C., 2000. An orally active anti-apoptotic molecule (CGP 3466B) preserves mitochondria and enhance survival in an animal model of motoneuron disease. Br. J. Pharmacol. 131, 721–728.

Serajuddin, A.T.M., Thakur, A.B., Ghoshal, R.N., Fakes, M.G., Ranadive, S.A., Morris, K.R., Varia, S.A., 1999. Selection of solid dosage form composition through drug-excipient compatibility testing. J. Pharm. Sci. 88, 696–704.

Waldmeier, P.C., Spooren, W.P.J.M., Hengerer, B., 2000. CGP 3466 protects dopaminergic neurons in lesion models of Parkinson's disease. Naunyn-Schmiedebergs Archives of Pharmacology 362, 526–537.